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Comparison of Phenotype in Uniparental Disomy and Deletion Prader-Willi Syndrome: Sex Specific Differences

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Prader-Willi syndrome (PWS) results primarily from either a paternal deletion of 15q11-q13 or maternal uniparental disomy (UPD) 15. Birth parameters and clinical presentation of 79 confirmed UPD cases and 43 deletion patients were compared in order to test whether any manifestations differ between the two groups. There were no major clinical differences between the two classes analyzed as a whole, other than the presence of hypopigmentation predominantly in the deletion group. However, there was a significant bias in sex-ratio (P<.001) limited to the UPD group with a predominance (68%) of males. An equal number of males and females was observed in the deletion group. When analyzed by sex, several significant differences between the UPD and deletion groups were observed. Female UPD patients were found to be less severely affected than female deletion patients in terms of length of gavage feeding and a later onset of hyperphagia. Although these traits are likely to be influenced by external factors, they may reflect a milder presentation of female UPD patients which could explain the observed sex bias by causing under-ascertainment of female UPD. Alternatively, there may be an effect of sex on either early trisomy 15 survival or the probability of somatic loss of a chromosome from a trisomic conceptus.

KEY WORDS: Prader-Willi syndrome, uniparental disomy, sex-ratio, trisomy 15

INTRODUCTION

The Prader-Willi syndrome (PWS) is defined to include delayed mental and psychomotor development, diminished fetal activity, severe infant hypotonia, hypogonadism, hypogenitalism, feeding problems in infancy, retarded bone age, characteristic face, small hands and feet, onset of gross obesity in early childhood (because of hyperphagia), short stature, a tendency to develop diabetes in childhood, and behavior problems [Prader et al., 1956]. Approximately 70–75% of PWS cases are caused by a paternal deletion of 15q11-q13 with most of the remaining cases showing maternal uniparental disomy [Nicholls et al., 1989; Butler, 1989; Robinson et al., 1991; Mascari et al., 1992]. A small subset of cases have been associated with imprinting mutations [Reis et al., 1994; Buiting et al., 1995]. The identical 15q11-q13 deletion occurring on the maternal chromosome results in the completely distinct constellation of traits known as Angelman syndrome (AS) [Butler et al., 1986, Knoll et al., 1989; Magenis et al., 1990]. Likewise, paternal UPD(15) is found in a small percentage of AS cases [Malcolm et al., 1991]. This region therefore provides one of the best known examples of genomic imprinting, i.e., the differential expression of genes depending on parent of origin.

There are several reasons why one might expect the clinical phenotype of deletions to be more severe than UPD. The typical PWS deletions are 45 Mb in size [Mutirangura et al., 1993] and may include many more genes than are imprinted. These non-imprinted genes would show half normal expression levels in deletion cases but not in UPD cases. In addition, there may be

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leaky expression of imprinted genes, such that the presence of two maternal copies can better compensate for the missing paternal copy. This theory was suggested by Bottani et al. [1994] to explain why paternal UPD(15) resulted in a milder phenotype in two AS patients when compared to deletion AS patients. A milder phenotype in AS UPD compared to deletion cases was also suggested by Gillessen-Kaesbach et al. [1995a].

Alternatively, additional abnormalities could be present in the UPD cases if there are imprinting effects for chromosome 15 loci outside the common deletion region. In addition, it is commonly thought that UPD may arise from trisomic zygote rescue, that is, rescue of a trisomic fertilization due to somatic loss of one extrachromosome during early development (for review, see Kalousek and Barrett, 1994]. Direct evidence for this mechanism leading to mat UPD(15) comes from observations of trisomy 15 in chorionic villus samples, but a normal karyotype (and UPD) in the fetus [Purvis-Smith et al., 1992; Cassidy et al., 1992]. Mosaicism with a trisomic cell line could result in a more severe phenotype in a subset of the UPD patients. Although the trisomic cell line is normally confined to the placenta, recent studies have shown that a high level of trisomy 16 in the placenta is associated with intrauterine growth retardation [Kalousek and Barrett, 1994].

A number of published reports have examined differences between deletion and non-deletion PWS patients. Those with a visible deletion have been reported as having a higher intelligence [Butler et al., 1986] and more frequent hypopigmentation of skin, hair, and iris compared to PWS patients without visible deletion [Butler, 1989]. However, these studies relied on cytogenetic techniques and may have misclassified deletion patients as non-deletion and vice-versa, as well as possibly including atypical patients who did not fulfill the molecular diagnosis of PWS. In the first comparison in which molecular data were used, Robinson et al. [1991] compared 7 disomy PWS patients with 21 deletion PWS patients and found no major differences between the two groups except for increased parental age in the UPD group. Subsequently, Gillessen-Kaesbach et al. [1995b] reported similar results with a much larger sample size. Here we report on clinical findings in an additional large group of known PWS uniparental disomy patients and compare them to those found in PWS deletion patients. These patients were additionally analyzed by subdividing groups by sex of the patient.

METHODS

Molecular diagnosis of UPD patients in this study was done through routine screening of PWS patients at seven contributing centers: Zurich (N=22), Essen (N=16), Vancouver (N=9), Munich (N=8), Greenwood (N=8), Jerusalem (N= 4), and Bethesda (N=12). DNA results were used to classify these 79 cases of maternal UPD(15) as resulting from either MI (n=59), MII (n=10), or mitotic (n=10) error. The data for classification of non-disjunction errors in many cases were reported previously [Robinson et al., 1993; Lerer et al., 1994]. The original seven UPD cases reported in Robinson et al. [1991] are included in this study, as are ten patients included in the Gillessen-Kaesbach et al. [1995b] clinical study. No clinical information other than sex of patient was available for any of the Bethesda patients and only sex and parental age was available from the Jerusalem patients. Thus clinical data in UPD cases was based primarily on data from five contributing centers. A group of 43 confirmed deletion patients was used as a control group. The deletion patients were primarily obtained from Zurich, with additional cases from Vancouver.

Clinical information was obtained retrospectively on subjects included in the study through completion of a standard clinical questionnaire [Robinson et al., 1991] by the referring clinical geneticist and/or by chart review. In many cases, only partial information was available and, as subjects were obtained from multiple centers, it was not possible for one clinician to see all patients. The age of patients ranged from birth to 26 years with an average age of 8.1 years in the disomy group and 10.6 in the deletion group. There was no difference in age between males and females within either group.

Clinical criteria analyzed included parental age, birth weight, birth length, reduced intrauterine activity, gavage feeding, neonatal hypotonia, weight, height, hypogonadism, cryptorchidism, hypopigmentation (fair hair relative to parents), strabismus, typical face, narrow forehead, indifference to pain, and age at onset of walking and hyperphagia. Other criteria were initially evaluated but there were not enough data to report. As clinical criteria were scored by the referring physician or from examination of medical records; therefore the criteria for some features, such as hypogonadism or presence of neonatal hypotonia may vary slightly depending on the observing clinician. Statistical analysis was performed using the Student's t-test and Fishers exact test.

RESULTS

Among the PWS maternal UPD patients, there were 54 males and 25 females (Table I). This difference from the expected 1:1 ratio was highly significant ($P<.001$). There was no male excess in the PWS deletion group (19 males and 24 females) and comparison of sex ratios between the two groups was also significant ($P<.01$). There were no statistically significant differences in sex of patients divided by contributing center or by stage of origin. However, we cannot exclude that the sex-bias is confined to meiotic errors, since the male to female ratios were 41 : 18, 8 : 2 and 5 : 5 for meiosis I, meiosis II and mitotic errors, respectively.

Parental ages and clinical data for the two groups are presented in Table I. Both the mean paternal and mean maternal age was significantly greater in the UPD group when compared to the deletion group. Deletion patients were found to be significantly more hypopigmented than UPD patients. An increased mean maternal age in the disomy group when compared to the deletion group confirms previous reports [Robinson et al., 1991, 1993; Gillessen-Kaesbach,1995b]. No other significant differences were found when males and females were analyzed together as one group.

A comparison between the two genetic classes subdivided by sex of the patient documented that disomy males were significantly shorter at birth when compared to deletion males ($P<.02$). Other birth and neonatal parameters examined did not differ. Female di-

TABLE I. A Comparison of Manifestations in UPD Verses Deletion PWS Patients

		n	UPD	n	Deletion	P
Mean paternal age (years)		49	35.8	29	30.4	<.00002
Mean maternal age (years)		55	33.2	33	27.3	<.00001
Sex ratio (Male:female)		79	54:25[a]	33	19:24	< .01
Birth weight (g) (mean)	Male	25	2745	10	2839	n.s.
	Female	15	2594	14	2430	n.s.
Birth length (cm) (mean)	Male	17	47.3	8	50.4	< .02
	Female	5	48.6	9	46.7	n.s.
Length of gavage feeding (weeks) (mean)	Male	17	4.2	9	7.9	n.s.
	Female	8	1.9	9	6.1	< .01
Onset of hyperphagia (years) (mean)	Male	9	3.3	6	2.1	n.s.
	Female	9	2.2	7	1.4	< .02
Onset of walking (years) (mean)	Male	5	2.6	6	2.5	n.s.
	Female	4	2.2	9	2.1	n.s.
Weight >95% for age (%)	Male[b]	10	90	9	88	n.s.
	Female[b]	6	33	13	61	n.s.
Reduced intrauterine activity (%)		27	74	24	83	n.s.
Neonatal hypotonia (%)		37	100	30	100	n.s.
Hypogonadism (%)		30	90	20	90	n.s.
Strabismus (%)		32	81	19	84	n.s.
Indifference to pain (%)		13	85	9	88	n.s.
Cryptorchidism (%) (male only)		24	83	12	83	n.s.
Narrow forehead (%)		19	84	21	100	n.s.
Typical face (%)		34	97	17	100	n.s.
Fair hair (relative to parents) (%)		18	39	39	77	< .01

[a] Comparison of sex-ratio to the expected 1:1 is significant at $P < 0.001$.
[b] Comparison of weight >95% between all males vs. females was significant ($P < 0.01$).

somy patients required a shorter course of gavage feeding ($P<.01$) and had a later onset of hyperphagia ($P<.01$) when compared to female deletion patients. This difference was not observed between the two male classes. With respect to birth parameters, averages for both genetic classes were significantly below expected norms. No other features examined were found to show statistically significant differences when either subdivided by sex or not. However, a comparison of all males (UPD and deletions) versus all females showed that significantly fewer females over two years of age were greater than the 95% in weight than were males ($P<.01$)

DISCUSSION

The data presented here confirm previous reports that there are no major significant differences between the PWS UPD and deletion groups except for frequency of hypopigmentation and increased maternal age [Butler, 1989; Robinson et al., 1991; Gillessen-Kaesbach et al. 1995b]. These data also did not show a difference in birth weight between the two classes which was suggested previously [Robinson et al., 1991; Gillessen-Kaesbach, 1995b]. This is in contrast to Angelman syndrome, whereby a milder phenotype has been reported to result from paternal UPD 15 when compared to deletion patients [Bottani et al., 1994; Gillessen-Kaesbach, 1995a].

Previous studies comparing clinical phenotype between deletion and non-deletion patients did not subdivide the data by sex of patient. However, a few significant differences were observed in the present study when the analysis is conditioned on sex, with female UPD patients showing results suggestive of a slightly milder presentation than deletion females. Specifically, female UPD patients showed shorter mean length of gavage feeding and later onset of hyperphagia when compared to female deletion patients (Table I). Although these are traits which may be strongly influenced by external factors such as parental behaviour, the tendency for less feeding problems in female UPD patients may reflect a less severe phenotype in this regard. Comparisons of weights above the 95th centile showed significantly less obesity in female than in male PWS patients with a tendency for female UPD patients to be less obese than female deletion patients.

A possible milder phenotype limited to female UPD patients could theoretically be related to the process of X inactivation in females. Imprinting has features in common with X inactivation and it has been suggested that some effects of autosomal imprinting may occur only in the presence of X-inactivation in females [Lubinsky and Hall, 1991]. This mechanism has been postulated to result in the discordance of Wiedemann-Beckwith syndrome in female monozygotic twins [Lubinsky and Hall, 1991; Clayton-Smith et al., 1992; rstavik et al., 1995]. Non-random X-inactivation was reported in one twin pair discordant for WBS [rstavik et al., 1995], However, we did not find evidence for a skewed X-inactivation pattern in a subset of six UPD females tested (data not shown).

The most striking finding of the present study is a highly significant excess of males in the UPD group (54 males and 25 females). A similar bias was not observed in the study of Gillessen-Kaesbach et al. [1995b] (26 males: 25 females); however, the present data confirm our earlier observation of excess males [Robinson et al., 1991]. Although in the Gillessen-Kaesbach study non-deletion patients included mechanisms other than UPD (i.e., imprinting mutations) this should have been a minor effect.

One possible explanation for the observed sex-ratio is that there is a bias in ascertainment, perhaps depending on the referring clinician, due to a milder phenotype in female UPD patients. These patients may be missed if they are not as obese nor as hypotonic as their deletion counterparts. There were no significant differences in sex-ratio between individual centers and all showed equal or excess males; however, the sample sizes were small when subdivided this way, and larger samples from each center would be necessary to determine if there is a clinician-dependent sex-dependent ascertainment bias.

Another possible explanation would be a sex-bias in *survival* of early trisomies. This would be consistent with our observation that the sex-bias was only observed for the meiotic errors. Interestingly, a significant excess of males has been previously reported in trisomic spontaneous abortions [Hassold et al.,1983]. Examining specifically chromosome 15 trisomy, we have compiled data from Hassold et al. [1983] (61 cases), Zaragosa et al. [1994] (17 cases) and trisomy 15 from B.C.s Childrens Hospital records (99 cases). The total numbers of males and females among these 175 trisomy 15 fetuses are 99 and 76, respectively. Thus, there is a (non-significant) excess of males, but this ratio of 1.3 does not appear to be as skewed as that observed in our PWS sample (2.2). Possibly there is preferential loss of one extra chromosome 15 in male trisomic fetuses leading to a higher rate of mosaicism in males. Non-mosaic (predominantly female?) trisomy 15 fetuses may be aborted very early in pregnancy and not ascertained as frequently either as late spontaneous abortions or in association with UPD.

As yet there are no clear answers, and it will be important to confirm with further studies whether the UPD sex ratio bias is due to either under-ascertainment of females or differential survival of male and female trisomic fetuses. The first mechanism would indicate that female UPD PWS patients are being substantially overlooked due to a milder phenotype, whereas the second would indicate that there is either some difference in survival of early trisomy 15 conceptuses or a possible difference in the probability of trisomic zygote rescue dependent on sex of the fetus.

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